Certificate of Analysis
Phosphate Sensor, 100 nmol

Part Number: PV4407
Lot Number: 2842731M
Immediate Storage: -80°C
Shipping Conditions: dry ice

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Description:

The Phosphate Sensor is a purified recombinant *E. coli* phosphate binding protein labeled on A197C with the environmentally sensitive fluorophore MDCC. Binding of inorganic phosphate to the Sensor is tight (K_d ~ 0.1 µM) and results in a large increase in fluorescence. The Sensor enables detection of phosphate in the high nanomolar to low micromolar range.

Concentration:

493 µM based on A280 (Extinction coefficient = 68,530 M -1 cm-1)

Signal Change:

≥ 14.1 -fold. The signal change is defined as the ratio of the fluorescence in the presence of excess phosphate to the fluorescence in the absence of phosphate. Measurements were captured on a Tecan Safire2™ plate reader with excitation at 430 nm (5 nm bandwidth) and emission at 450 nm (5 nm bandwidth). Reactions contained 417 µM Sensor alone or with 1.7 mM phosphate. The signal change will be highly dependent on instrument settings and specific instrument used.

Dye to Protein Ratio:

≥ 0.95

Storage and Handling:

Store at -80°C.

Storage Buffer:

10 mM Tris-HCl (pH 7.6), 50 mM NaCl

QUALITY ASSURANCE

Phosphate Background:

0%

Phosphate background is calculated by the following equation:

Phosphate background = $(F_a - F_m) / (F_p - F_m)$

F_a = fluorescence of the Sensor alone.

F_m = fluorescence of the Sensor in the presence of the phosphate mop (see below).

F_p = fluorescence of the Sensor in the presence of excess phosphate.

Purity:

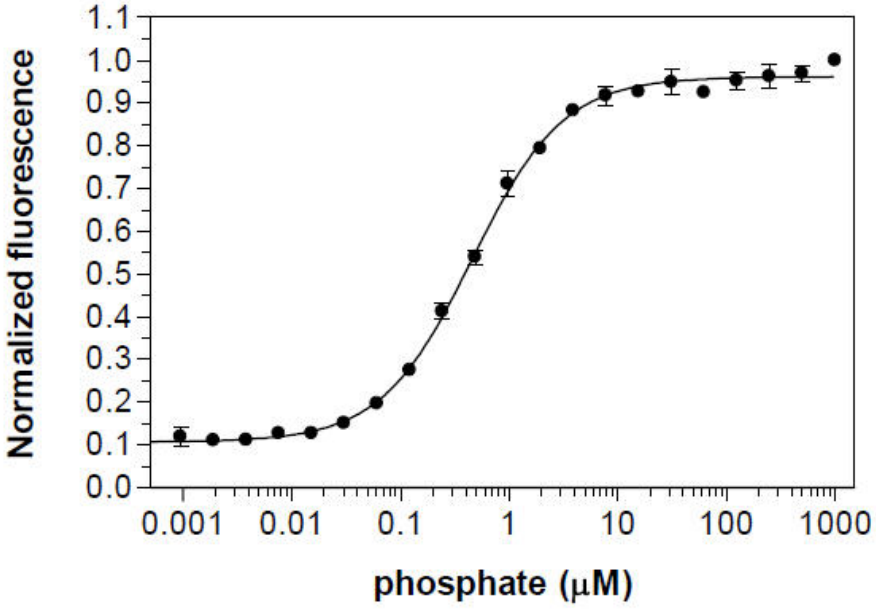
95% as determined by a SDS-PAGE gel stained with SimplyBlue™ SafeStain.

Molecular Weight:

35 kDa.

USAGE

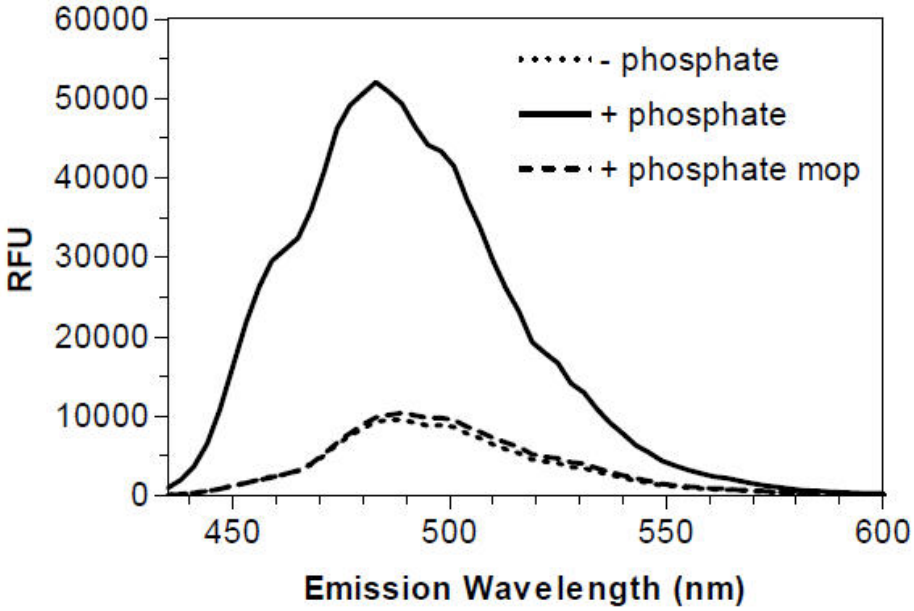
Phosphate Sensor Standard Curve:



A representative phosphate standard curve is displayed using the recommended concentration of 0.5 µM phosphate sensor. 10 µL of Sensor (in 20 mM Tris pH 7.6, 0.05% Triton X-100) was added to 10 µL of phosphate standard in a black low volume, uncoated 384-well plate (Corning part# 3677). Care should be used when selecting a microplate as some surface-coated plates have been shown to contain significant amounts of phosphate.

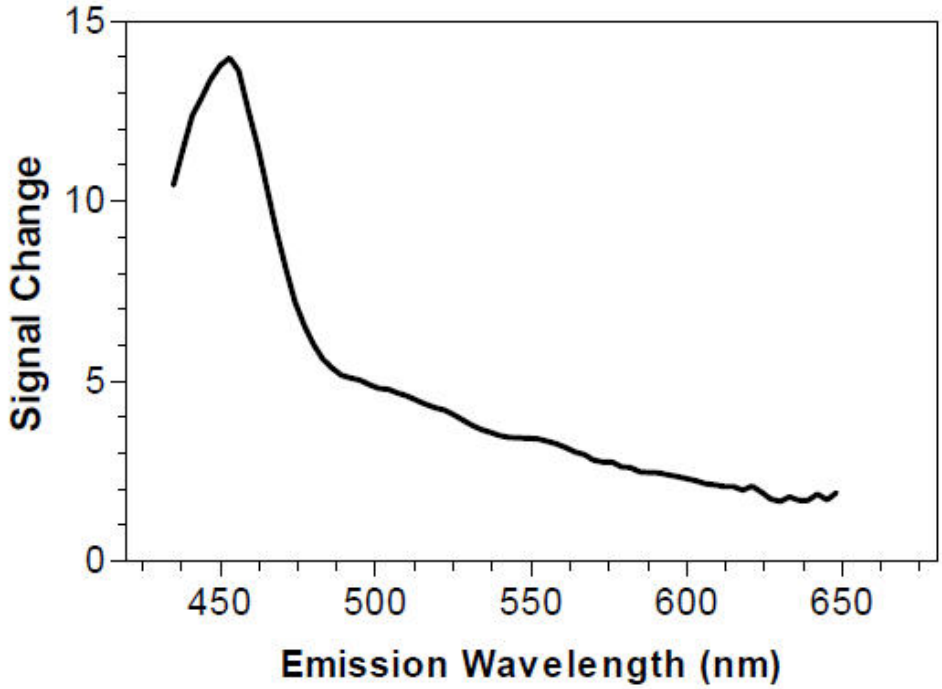
Fluorescence measurements were captured on a Tecan Safire^{2}™ plate reader with excitation at 430 nm (10 nm band width) and emission at 450 nm (10 nm band width). The sigmoidal dose-response (variable slope) equation [Y=Bottom + (Top-Bottom)/(1+10^((LogEC50-X)*HillSlope))] was fit to the data using GraphPad Prism® software. This curve fit can be used to convert fluorescence data from unknowns to actual phosphate concentrations.

Emission Spectra:



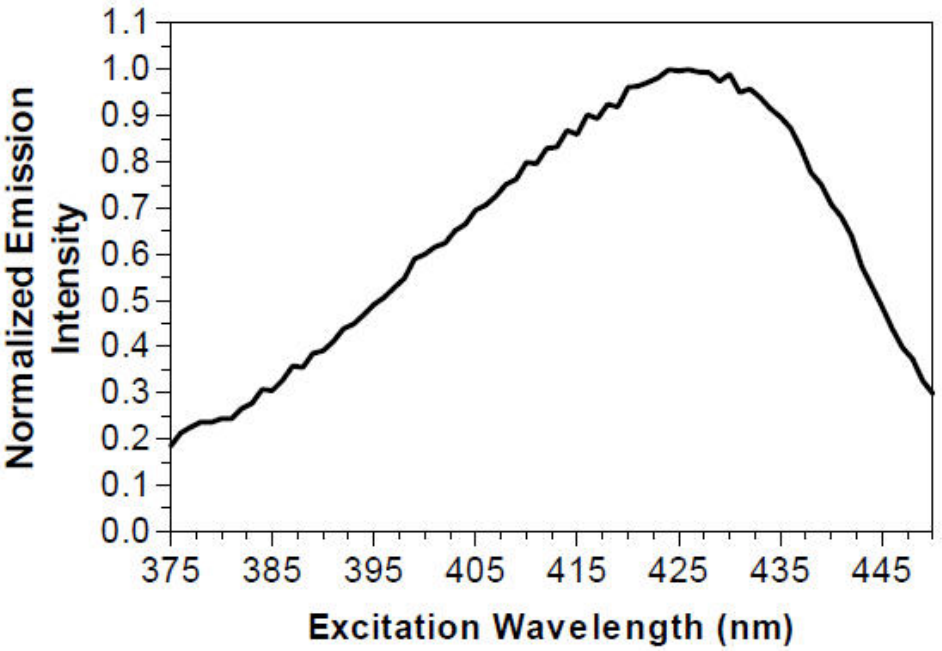
Emission spectra of a representative lot were captured at 417 µM Sensor alone, with 1.7 mM phosphate, and with phosphate mop (200 µM 7-methylguanosine and 0.2 U/mL Bacterial Nucleoside Phosphorylase). The phosphate mop sequesters any inorganic phosphate in the form of ribose-1-phosphate. Fluorescence measurements were captured on a Tecan Safire^{2}™ plate reader with excitation at 430 nm (5 nm band width) and emission at 450 nm (5 nm band width).

Optimization of Emission Wavelength:



The signal change was determined across a range of emission wavelengths. The largest signal change occurs at emission wavelengths near 450-460 nm, which is relatively close to the recommended excitation wavelengths of 420-430 nm. Thus, for filter-based instruments a narrow band width filter is recommended at 450 – 460 nm. Omega Optical filters 420BP10 and 460BP10 have been used successfully. However, emission wavelengths of 465 to 480 nm may provide a robust signal for many applications. Optimal settings will depend on the specific instrument used.

Excitation Spectrum:



Fluorescence intensity was determined across a range of excitation wavelengths. Maximum excitation occurs at approximately 425 nm. However, optimal excitation settings will be dependent on the selected emission wavelength and bandwidths. Suitable wavelengths may be in the 390 to 430 nm range.

Phosphate Mop:

When using this product care should be taken to minimize contamination from other reagents as phosphate is common in biological materials and on glassware. Inclusion of the phosphate mop (1, 3) in control samples can be useful to identify sources of contaminating phosphate or rule out contamination as a potential problem.

The phosphate mop is comprised of 7-methyl guanosine (7-MEG) and purine nucleoside phosphorylase (PNPase) and sequesters inorganic phosphate in the form of ribose-1-phosphate. 200 µM 7-MEG and 0.1 to 1.0 U/mL PNPase are used for typical applications. 7-MEG can be dissolved in water to 30 mM and stored at -80°C. PNPase can be dissolved in water to 500 U/mL and stored in small aliquots at -80°C (avoid freeze/thaw cycles).

Care should be used when selecting a microplate as certain surface-coated plates have been shown to contain significant amounts of phosphate. Corning 384-well uncoated plates (#4511) plates are recommended, though other coated and uncoated plates have also been used successfully.

Chevohn Joseph, Director, Quality Date: 16/Nov/2023